Short-term movements and behaviour govern the use of road mitigation measures by a protected amphibian

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Abstract

Road mitigation infrastructure for pond-breeding amphibians aims to provide a safe and sustainable passage for individuals between critical habitat patches. However, relatively little is known about how amphibians interact with mitigation systems because of the challenges of documenting movements at sufficiently large sample sizes. The effect of real or perceived barriers to short-term movement could ultimately determine the success or failure of road mitigation schemes. We quantified behavioural responses of the protected great crested newt (*Triturus cristatus*) in a complex road mitigation system in the UK. We used fluorescent paint to mark individuals in order to measure distance travelled and trajectory orientation over two seasons (spring when adults migrate to breeding ponds and autumn when newts disperse) and in three components of the mitigation system (fences, tunnel entrances and inside the tunnels). A total of 250 juveniles and 137 adult great crested newts were marked and tracked during 38 survey nights. Adult newts were individually identified using belly-pattern recognition. There was substantially greater activity along the fences during autumn (82% of newt captures) compared to spring. *Triturus cristatus* typically moved short distances each night (3.21 m night^{-1} in spring and 6.72 m night^{-1} in

autumn), with a maximum of 25.6 m travelled inside a tunnel. Adult recapture rates were low (9.7%) and only 3% of the newts found along the fences reached the tunnel entrances. Movements were straighter in spring and inside the tunnels and newts had higher crossing rates in autumn compared to spring. Overall, behaviour and seasonal movement patterns significantly influenced the use of the mitigation system, in a way that could impact landscape connectivity for *T. cristatus* over the long-term. Adequate incorporation of fine-scale movement dynamics could help develop new behavioural models, inform our understanding of amphibian ecology and substantially improve future road mitigation projects.

Keywords: connectivity, dispersal, *Triturus cristatus*, individual behaviour, migration, underpasses.

Introduction

Roads cause multiple and diverse ecological impacts on habitat and wildlife populations by directly contributing to habitat loss and reducing the quality of surrounding habitats (Forman & Alexander, 1998; Spellerberg, 1998; Trombulak & Frissel, 2000). The negative influence of roads threatens population viability through wildlife traffic-related mortality and by acting as a barrier to animal movements (Forman et al., 2003), therefore increasing habitat fragmentation and isolation.

For amphibian populations, habitat loss and consequent fragmentation represent major threats (Cushman, 2006). Road construction in particular reduces habitat availability and increases the distance between habitat patches that are crucial for seasonal movements and maintenance of population dynamics for amphibians (Fahrig et al., 1994; Hels & Buchwald, 2001). Moreover, high amphibian mortality rates on roads and road avoidance behaviour compound these impacts, leading to worldwide concern about the potential effects of roads on amphibian population viability (Fahrig et al., 1994; Jaeger and Fahrig et al., 2004; Glista, deVault & DeWoody, 2007; Petrovan & Schmidt, 2016).

Road mitigation measures such as tunnels and fences have been implemented for decades, aiming to safely guide amphibians between habitat patches in order to maintain landscape-scale connectivity within and between populations (Beebee, 2013). Knowledge of terrestrial movement patterns is typically used to guide decisions on the locations of mitigation systems (Clevenger & Waltho, 2005). The protected great

crested newt (*Triturus cristatus*) is one of the main road mitigation target species in Europe (Ward, Dendy & Cowan, 2015; Matos et al. 2017), and landscape permeability is essential for the species' dispersal and migration (Halle, Oldham & Arntzen, 1996; Griffiths, Sewell & McCrea, 2010). After implementation of road mitigation, great crested newts can be particularly active around tunnels and fences, especially after emigration from ponds to hibernation and refuge sites during autumn (adults and juveniles) and during spring migration when adults move towards the ponds to reproduce (Matos et al., 2017) yet detailed aspects of their interactions with the mitigation system remain unknown. Previous studies on amphibians suggest that fences and tunnels alter typical behaviour and that responses to mitigation infrastructure are species specific (Jackson & Tyning, 1989; Allaback & Laabs, 2003; Pagnucco, Paszkowski & Scrimgeour, 2011). Post-mitigation monitoring often inadequately assesses mitigation effectiveness and typically does not account for the behaviour of target species at different points along the mitigation system (Woltz, Gibbs & Ducey, 2008, Pagnucco, Paszkowski & Scrimgeour, 2012). For example, no evidence is available to explain whether pond-breeding amphibians use mitigation systems as part of their ranges or whether they simply use them for transit (Langton, 1989; Hamer, Langton & Lesbarrères, 2015). Similarly, it remains unclear whether amphibian fences actively guide amphibians towards tunnels or rather deflect their movements at random. Mitigation advice assumes that amphibians will follow fences for considerable distances

(30-50 m or more) in order to reach tunnel entrances (Schmidt & Zumbach, 2008) yet this is largely untested, especially for newt species. Even when amphibians encounter tunnels, they may be reluctant to enter and cross them (Jackson & Tyning, 1989; Allaback & Laabs, 2003; Matos et al. 2017).

Quantitative analyses of behavioural changes in movement patterns in response to road mitigation (eg. attraction/rejection, distances moved and trajectory orientation) are extremely limited. Studies describing encounter and transit of each part of the mitigation system by newts during critical phases of the annual cycle are lacking (Schmidt & Zumbach, 2008; Hamer et al., 2015), limiting opportunities for evidence-based improvements to mitigation systems. Understanding the type and the consequence of movements performed by individuals could inform individual-based models for predicting population-scale movements relative to mitigation systems and thus enable more effective advice on fence and tunnel placement (Pontoppidan & Nachman, 2013). However, individual behaviour among amphibians guides their dispersal success and can influence populations' distribution patterns in the landscape (Baguette & Dyck, 2007, Sinsch, 2014). Newts display a range of behaviours and motivations while moving on land (e.g. foraging, searching for refuge, use of underground shelters), and slight changes of direction for each individual can lead to different conclusions on the intent of the movement (i.e. dispersers or residents) (Sinsch, 2014). Traditional techniques for studying terrestrial movements of urodeles

can directly influence their behaviour thus confounding conclusions about individual choices. For instance, radio-tracking has been used to study terrestrial movements of *Triturus* species (Jehle & Arntzen, 2000, Schabetsberger et al., 2004; Jehle, 2010, Jehle, Thiesmeier & Foster, 2011) providing information on total distance travelled (30 – 400 m) and direction of movement after and before the aquatic phase. Yet, transmitters must be internally implanted, force-swallowed or externally mounted, with consequences for behaviour (Schabetsberger et al., 2004). Moreover, the number of newts that can be efficiently followed over a short period of time using this technique is restricted and limited in spatial scale (1-20 m) (Jehle, 2000).

This study aimed to investigate the short-term terrestrial movements of individual *T. cristatus* within a road mitigation system. We tested the following assumptions about the great crested newt's use of the mitigation system: (1) On contact with a fence, newts adjust their movement to follow it; (2) On encountering a tunnel entrance newts move into and cross through the tunnel; (3) Activity peaks in the annual cycle in spring (migration) and autumn (dispersal) are reflected differently in the use of the mitigation system.

For this, we measured distance travelled and trajectory orientation of individual *T. cristatus* per night during two field seasons, in autumn and spring. In addition, we quantified the proportion of recaptured *T. cristatus* and their final positions in the system using photographs of adult belly-patterns.

Materials and Methods

Study site

The study was conducted at Orton Pit/Hampton Nature Reserve, Cambridgeshire, UK (52º 32'24N, 0º16'53W) (**Fig 1a**), a 145ha Site of Special Scientific Interest, a Special Area of Conservation and a Natura 2000 site. The area is characterised by a complex of over 340 ponds, between 15-50 years old. The site is currently home to the largest known single population of great crested newts in the UK and possibly Europe, estimated at around 30,000 individuals (JNCC, 2015) as well as a very large population of smooth newt (*Lissotriton vulgaris*), and small numbers of common frog (*Rana temporaria*) and common toad (*Bufo bufo*).

Between 1990 and 2000 a large-scale habitat restoration programme was implemented to protect the great crested newts, including pond modification and fish eradications. In 2006, a new housing development was built towards the north of the reserve, serviced by a 10-12m wide road designed to support 1000 to 10 000 vehicles per day. Concrete 'newt barriers' were installed adjacent to this road (**Fig. 1b**). During road construction some large ponds were re-profiled and some were in-filled to accommodate the road. A mitigation system was installed to facilitate movement of amphibians under the road between the eastern and western parts of the reserve (**Fig. 1b**).

The road mitigation system was composed of one 0.5 m diameter, 30 m long polymer concrete 'amphibian tunnel' with open slots at the top

(ACO, Germany) in the centre, two large ARCO concrete and metal sheet underpasses (5.5 m wide x 2 m high, 40 m long) spaced 100 m apart and two 200 to 300 m long heavy duty plastic fence systems (Herpetosure UK), placed 10-50 m away from the road and partly angled (~45º) to guide amphibians towards the tunnels. The distance from the tunnels to the nearest great crested newt breeding pond is at least 31-74 m depending on tunnel and side of the road. In an effort to minimise human disturbance the entrances of the large tunnels were protected with a bar fence and gate system.

Data collection

T. cristatus movement data were collected during two periods of peak activity: autumn (between 17 September and 26 October 2014 covering the period of post-breeding dispersal) and spring (between 6 March and 3 April 2015, covering the period of adult migration towards breeding sites). For both seasons, night surveys (marking) started 2-3 h after the sunset and had a mean duration of 2 h. Morning surveys (recaptures) started 1 hour before sunrise and mean duration depended on the number of *T. cristatus* captured (2-3 h). Days with favourable weather (rain in the last 3 days and temperatures above 6ºC) were specifically selected to maximise the number of *T. cristatus* monitored.

Tracking method

We used multiple fluorescent pigment applications to track the short-term movements of *T. cristatus* throughout the road mitigation system. Fluorescent pigments have been proven a useful technique in microhabitat studies for amphibians (Eggert, 2002; Ramirez et al., 2012). The extent of daily movements might be over-estimated by home range analysis (Wells, 2007); however, fluorescent marking can provide detailed information on daily patterns, even under wet conditions, and especially for newts because of their relative short movements compared to other amphibians (Jehle, 2000). Although it has been used in combination with transmitters to improve detectability, pigments alone, when rapidly applied, do not seem to influence behaviour (Eggert, 2002). Once painted and released, newts leave a discrete fluorescent trail that can be observed for several hours or even days, whilst avoiding the need for more invasive and disruptive tracking methods. Using this method, a single observer can monitor the movements of hundreds of individuals, collecting detailed information on trajectory orientation and type of movement displayed. This method has been tested on a range of amphibians at different life stages and is considered safe and less evasive than radio tracking for assessing short-term movements (Nishikawa, 1990; Eggert, 2002; Roe & Grayson, 2009; Pittman & Semlitsch, 2013).

T. cristatus capture-mark-recapture

Newts were surveyed by following fences along both sides of the road during each survey with the start point alternating between surveys (A in **Fig. 1b**). Newts were also surveyed at tunnel entrances (within 2-3 m radius around tunnel openings: **Fig. 1b**) and inside the tunnels (north and south tunnels: C in **Fig. 1b**). Only the two large tunnels permitted entry to capture individuals and measure behaviour inside the tunnel.

At initial observation, individual activity and position in the system (at the fence, tunnel entrance and inside the tunnel) were recorded. Orientation and microhabitat use at the start and end of movement, as well as activity in three specific categories (moving, foraging, and stationary) were also documented. Adult great crested newts were captured by hand, given a unique identification number, and a photograph was taken of the ventral pattern for future identification. Life stage (adult/juvenile) and sex (female/male) were recorded, and a colour was allocated for marking. Marking consisted of dipping newts' tails, hind legs and ventral surface in fluorescent powder (DayGlo Color Corp. fluorescent pigments) and pressing gently to ensure adhesion of sufficient powder. Newts were marked with four different colours (Z-18-3 Green, Z-15-3 Orange, Z-17-N Yellow and Z-11-3 Pink) in order to differentiate paths within and between surveys (**Fig. 2a**).

To minimize stress, animal processing was completed in less than 1 minute and no body measurements were collected. Newts were released at their exact point of capture and left undisturbed for at least 5 h. We used different colours to mark individuals in close proximity and

removed paint tracks from previous nights with a brush or by disturbing the soil before each survey.

To evaluate minimum long-term movements between seasons and positions in the system we used ventral patterns to identify recaptured adults. Recaptures were identified automatically from photographs using I3S software (pattern recognition software) (Hartog & Reijns, 2014). Recapture rates were calculated by season, position in the system and between initial and final positions.

Position recording

T. cristatus trails were monitored using an ultraviolet light (UV WF-501B LED 365NM; Ultrafire Official Genuine Shop, Guangdong, China) to detect the fluorescent powder. Trail start/end positions and points at each directional change were temporarily marked with small flags and distances were recorded using a tape measure (to the nearest 0.01m). Track trajectory was measured using a compass (using a range of 0-180º) in relation to the position in the system (see below Data analysis). Final location was recorded to the nearest 3m using a portable GPS (GPS Essentials 4.4.8, 2015). If the entire path was less than 30-40 cm between the initial capture point and the final point, movement was recorded as zero (similar to Ramirez et al., 2012).

Data analysis

Short-term movements were analysed using data on distance travelled, track trajectory and frequency of movement performed by adults and juvenile *T. cristatus* in three positions along the road mitigation system (fence, tunnel entrance and inside the tunnels) and between seasons (autumn and spring). For simplification, only two age classes were used: adults and juveniles. The latter comprised both young-of-the-year in autumn as well as individuals hatched in the previous year.

Movement data: distance travelled and trajectory orientation

Movement data for each individual were analysed as total distance travelled per night (track length) and track trajectory. Track trajectory was summarised as straightness and orientation relative to the mitigation system. Straightness was calculated from the ratio between displacement and total distance travelled (**Fig. 2b**). Displacement distance was determined by dividing the straight-line distance between the start to the end of track with total distance travelled (Sinsch, 1990; Roe & Grayson, 2009). Straightness is an index that varies between 0-1; values close to 0 indicate a curved route and a value of 1 indicates a straight path. This analysis can distinguish between rambling behaviour and directed movements. Orientation was calculated using the deflection angle at three capture points (**Fig. 2b**). When individuals were captured at the fences or tunnel entrances, angles ranged between 0º and 180º. When captured inside the tunnels, angles ranged between 0º and 90º. Inside the tunnels and along the fences, values of 0º indicated individuals moving

towards the exit/entrance of the tunnel and values of 90º-180º indicated individuals moving away from entrance/exit of the tunnel.

Season and location effects

Movement data of adults and juveniles that moved > 40 cm per night were summarised by season (autumn and spring), location inside the mitigation system and life stage. To quantify differences in behaviour in different parts of the system, we calculated: (1) the number of individuals moving from the three positions in the system (fence, tunnel entrance and inside the tunnel) and final positions (surrounding environment or remaining in the system) and (2) changes in distance travelled and orientation relative to the three positions in the system. We used a generalized linear mixed model (GLMM) to calculate the effects of season, position in the system and life stage on movements of *T. cristatus*. Season, position and life stage were fixed effects and position and life stage were nested within season, which was entered as a random effect. Three null models containing the most significant variables and intercept were included for comparison (season + mitigation + life stage + (1|season)). We then tested three models with the most significant variables, with no test for interactions. We compared model fit using Akaike information criterion (AIC) to optimize goodness-of-fit but avoided overfitting of the candidate models (Burnham & Anderson, 2002). After selecting the most parsimonious model, we determined the significance of fixed factors by analysis of

deviance (Burnham & Anderson, 2002). Models were fitted using a ML distribution and lmer function in package lm4 (Bates et al., 2014) in R software (R Core Team, 2016).

Results

In total, 38 surveys were carried out: 24 during autumn and 14 during spring. Cold and dry weather meant that additional surveys in spring had to be abandoned as no newts could be found. A total of 387 *T. cristatus* were caught and paint-marked (280 and 107 per season, respectively); of these, 250 were juveniles and 137 were adults (73 females and 64 males) (**Table 1; Table S1**).

Among the 270 (70%) *T. cristatus* that moved >40 cm, 44 (16.3%) were adult males, 35 (13%) adult females and 191 (70.7%), were juveniles), a higher percentage moved in autumn (82%) than in spring (18%, **Table 1**). Most movements were performed along the fences during autumn (58%) with no *T. cristatus* found inside the tunnels in spring. Final positions were mainly recorded in the mitigation system (n = 215, 80%) while 55 (20%) were found moving in the direction of the surrounding environment (**Table 1**).

Final positions and seasonal movements

For both seasons combined, only 3% of newts moving along the fences encountered the tunnels (3 adults and 3 juveniles), 25% moved to the surrounding environment (23 adults and 26 juveniles), and approximately 70% remained at the fences (53 adults and 89 juveniles). When captured at the tunnel entrances, the majority of *T. cristatus* moved towards the fences (70%), 11% moved in the direction of the surrounding environment and only 19% stayed at the entrance or entered the tunnel.

A high percentage of *T. cristatus* captured inside the tunnels remained inside or at the entrance (78%) while 22% found the tunnel exit towards the surrounding environment (4%) or reached the fences on the opposite side of the road (18%) (**Fig. 3**).

The adult recapture rate was small and not significantly different between seasons, averaging at 9.7% (**Table 2**). Number of recaptures were borderline significantly different between locations in the system (χ^2= 5.94, df = 2, P = 0.051). Most recaptured *T. cristatus* were located around the fences (11%) and 3% were captured in the tunnels (entrance and inside). The majority of recaptures was at the fence (4%), followed by a small percentage in the tunnels (1%) and surrounding environment (0.5%).

Distance travelled

The vast majority of *T. cristatus* moved very short distances, with 77% of those tracked during autumn and 97% in spring moving less than 5m per night (**Fig. 4; Fig. 5**). Distances of more than 10 m per night were only recorded in autumn (5% of juveniles and 1% of adults).

Variation in distance travelled per night was dependent on the initial capture position in the system (**Table** 3). There was a higher number of *T. cristatus* at the fences (n= 197) with shorter movements (mean per night = 3.21 m), a low number of captures at the tunnel entrances (n= 45) with short movements (mean per night = 3.41 m), and a small number of

captures inside the tunnel (n=28) with long movements (mean per night = 6.72 m) (**Fig. 5; Table 3**).

The maximum distance travelled in a night was 25.6 m (adult male) inside a tunnel. For adult recaptures, the greatest distance between capture points was 20.3 m by a female over 38 nights.

Trajectory orientation

Newts were observed moving in different directions in relation to the fences; no particular angles were preferred (**Fig. 5**). There was a significant effect of season on directionality (straightness of 1), with *T. cristatus* paths being straighter during spring (**Table 4**). Orientation of trajectories was dependent on individuals' position in the system. Trajectory orientation was significantly different for newts moving along the fences compared to the newts moving around the tunnel entrances and inside the tunnels, with a clear orientation towards the tunnel entrance or exit apparent inside the tunnels (**Fig. 5; Table 4**).

Discussion

Using fluorescent marking to investigate fine-scale movement of *T. cristatus* we showed that: (1) distances travelled per night were generally small; (2) very few *T. cristatus* moving along mitigation fences reached and entered the road tunnels; (3) *T. cristatus* moving into tunnels remained there at least overnight but their movements were straighter than outside; (3) the orientation of movements at the fences was mostly away from the tunnels (4) there were large seasonal differences in movements (longer movements during autumn compared to spring).

Behaviour along the fences

Despite the high number of *T. cristatus* encountered at the fences during the night, the observed low adult recapture rate between seasons and the small capture numbers in spring suggests that individuals do not cross the road as part of annual breeding migrations. Additionally, few encounters with the tunnel entrances and the straight paths at a range of angles away from the fences are consistent with *T. cristatus* using the fences as part of their normal foraging environment (Oldham et al. 2000). Fences may constitute barriers, keeping newts away from the road, hence preventing road mortality, but do not appear to guide *T. cristatus* directly towards tunnels. This is consistent with previous results that showed salamanders moving in different directions along the fence and not specifically towards the tunnel entrances (Allaback &

Laabs, 2003). Effective tunnel crossings by newts may therefore be improved by positioning more tunnels close together. Allaback & Laabs, (2003) suggested a maximum of 30 m intervals between tunnels for salamanders, yet our results suggest that such distances are too great for *T. cristatus*.

For amphibians that can suffer high road mortality such as the common toad *Bufo bufo*, fences can offer greater benefits by reducing mortality and directly guiding individuals towards tunnels (Lesbarrerès, Lode & Merila, 2004). Given the lower terrestrial movements of newts (Kovar et al., 2009) along with our results showing a tendency to reflect off the fence in random directions, this may mean that fences result in less frequent road crossings and hence lower environmental connectivity. Furthermore, the fence area offered good refuge conditions; newts use small mammal burrows (Jehle & Arntzen, 2000), which were abundant along the fences. Active feeding and refuge use was observed during this study, especially in areas with dense vegetation.

Behaviour in the tunnels

Our results indicated differences in distances travelled and orientation of *T. cristatus* at the entrance and inside the tunnels. At tunnel entrances, distances travelled by *T. cristatus* were shorter than inside the tunnels. A higher proportion of animals might be guided towards the tunnels if fences adjacent to the entrance were angled by 45º in relation to the tunnel and shorter than 20 m (Jackson, 2003). Entrances in this study

presented the same angle (45º, see Fig. 5) and were 3 m long. Nevertheless, *T. cristatus* seemed to change path where the fence angle at the tunnel entrance changed to direct them towards the inside of the tunnel and numbers of individuals attempting to cross were still very low (20%). Experimental studies could test the optimal length of fence at the tunnel entrances in order to maximise opportunities for encountering the tunnel entrance.

The majority of *T. cristatus* found at the tunnel entrance moved away from the tunnel and towards the fence or the surrounding environment. Changes in orientation in tunnel entrances have been reported for *Ambystoma* species (Jackson & Tyning, 1989; Allaback & Laabs, 2003). Microhabitat conditions at tunnel entrances can impact the way in which amphibians move through this area to get to the tunnel (Glista, DeVault & DeWoody, 2009) and amphibian tunnels can suffer significant pollution from the road surface (White, Mayes & Petrovan, 2017). Usually tunnel entrances are cleared of vegetation and debris to facilitate entry by amphibians, but that can create areas denuded of vegetation (Langton, 1989). Newts may hesitate to enter tunnels due the changes in microhabitat and environment (e.g. temperature and moisture) associated with vegetation removal (Allaback & Laabs, 2003). Cover may determine if a tunnel may be more ‘attractive’ and natural vegetation could provide a continuity of habitat towards and inside the tunnel (Glista et al., 2009). It is still unclear whether *T. cristatus* choose a particular environment in

these systems or if they return to known refugia or other environments while moving on land during the night.

Once inside the tunnel, *T. cristatus* tended to move in straight paths towards the tunnel exit but percentages of individuals making a full or partial (if the individual was found already inside the tunnel) crossing in one night were low. In past *ex situ* experiments, low rates of tunnel crossing rates were recorded - 0.68 - 0.77, 0.27-0.66 and 0.07–0.21 (Lesbarrerès et al., 2004; Woltz et al., 2008, Hamer et al., 2014, respectively). Tunnel use in this study was higher for *T. cristatus* over both seasons (0.8 captures per night). Numerous factors appear to influence the decision of an amphibian to enter and cross a tunnel (Glista et al., 2009), e.g. position of system, moisture, temperature, light, substrate and noise and the understanding of how tunnels can be adapted to meet the best environmental conditions for full successful crossings is still improving (Lesbarrerès et al., 2004; Patrick et al., 2010).

The low number of adult *T. cristatus* moving towards the tunnels combined with changes of orientation and the small number of recaptures suggest that responses towards human-made infrastructure may be negative (Jehle & Arntzen, 2000; Knowlton & Graham, 2010). Adult *T. cristatus* prefer areas well covered with vegetation around breeding ponds and show a strong sense of fidelity towards breeding sites (Jehle, 2000). This fidelity may be extended to the terrestrial sites around ponds, causing adults to remain near the pond between breeding seasons (Sinsch, 1990, 2014; Jehle et al., 2011). Due to homing ability, adults

perform shorter and less random/explorative movements when encountering a fence or a tunnel, which may play an important role in the changes of orientation and non-permanence in the system (Sinsch & Kirst, 2015). However, information on amphibian habituation and evidence on use of navigation cues and homing to explain behaviour at the entrance and inside the tunnels is still lacking (Pagnucco et al., 2012). Overall the effectiveness of tunnels in facilitating successful crossing by amphibians may be linked to how such infrastructure is sensitive to behavioural changes and inter-species differences (Baguette & Dyck, 2007).

Seasonal effect in movements

Differences in frequencies of use of the mitigation system and movement length between the two seasons may illustrate the type of movement that the system facilitates (Sinsch, 2014). The longer and more frequent movements in autumn compared to spring are consistent with dispersal being the main type of movement facilitated by the system in the present study (Matos et al., 2017).

Although distances travelled were shorter in spring, there was an even stronger effect of season on the path straightness of *T. cristatus*. Spring migration is typically considered to involve more direct, straight-line movements (Jehle & Arntzen, 2000; Wells, 2007) but that was not the case in our study. The number of spring days with precipitation was

lower than in autumn, which might have influenced the straightness of movement patterns (Kovar et al., 2009).

Conclusions and future research

Our findings highlight three important aspects of road mitigation systems that may influence *T. cristatus* behaviour and consequently the effectiveness of these systems: 1) mitigation systems need to support the longer-distance and more frequent movements in autumn by facilitating tunnel crossing and metapopulation connectivity; 2) fences generally acted as barriers to movement, directing *T. cristatus* away from the road and rarely towards the tunnels; 3) overall tunnel use was low and *T. cristatus*' responses to encountering the tunnel (change in orientation) rarely resulted in tunnel crossing. A better understanding of the consequences of these results for metapopulation dynamics and persistence, as well as behavioural responses of *T. cristatus* to infrastructure could help determine if improvements to tunnel design are required.

Behavioural data collection is time consuming but once compiled it can be used to parameterise individual-based or random walk models to simulate long-term amphibian movements (Pontoppidan & Nachman, 2013; Pittman Osbourn & Semlitsch, 2014; Sinsch, 2014). In combination with data on population dynamics, individual-based modelling could provide insights into the minimal number of successful crossings per year for population persistence over the long-term (30-50

years). The adequate inclusion of behavioural data in road ecology studies has the potential to generate critical evidence for improving the effectiveness of such schemes as well as increasing our understanding of limits and motivations in movement ecology for different species in a changing environment.

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Table 1 – Movements of adult and juvenile *T. cristatus* marked with fluorescent powder in autumn 2014 and spring 2015.

	Season					
	Autumn			**Spring**		
Distance (m)	**max**	**min**	**median**	**max**	**min**	**median**
females	16.4	0.6	2.72	6.5	1.0	3.0
males	25.6	0.5	2.52	7.7	1.5	2.9
juveniles	19.4	0.6	3.1	5.3	1.3	2.2
Straightness (0-1)						
females	1.0	0.0	1.0	1.0	0.65	1.0
males	1.0	0.33	1.0	1.0	0.64	1.0
juveniles	1.0	0.0	1.0	1.0	0.51	1.0
Orientation (0º-180º)						
females	180.0	0.0	84.38	180.0	0.0	30.0
males	180.0	0.0	90.00	180.0	0.0	180.0
juveniles	180.0	0.0	90.00	180.0	0.0	180.0

Table 2 – Movement parameters for adult *T. cristatus* recaptured by sex (females and males).

Recaptures	**Females**	**Males**
nº points	31	25
nº indiv	14	10
moved	11	9
distance (m)	0.8-16.4	1.5-11.8
straightness	0.33-1.0	0.33-1.0
orientation (0º-180º)	17.2-112.5	22.5-157.5

Number of position recapture points, number of individuals that moved, and range of values for distance travelled, straightness and orientation per night.

1 Table 3 – Mean value and range of observed behaviour per season and position in the mitigation system

Variables	**Season** mean (range)		**Position in mitigation** mean (range)		
	Autumn (n=144)	Spring (n=86)	Fence (n=76)	Tunnel entrance (n=76)	Tunnel (n=76)
Distance travelled per night (m)	4.20 (0.50-25.60)	2.81 (1.30-4.90)	3.21 (0.5-15.3)	3.41 (1.1-8.65)	6.72 (0.75-25.60)
Straightness (0-1)	0.84 (0-1)	0.95 (0.77-1.0)	0.89 (0.17-1.0)	0.83 (0.42-1.0)	0.78 (0.0-1.0)
Orientation (0-180)	77.02 (0-180)	109.5 (0.0-180.0)	93.81 (0.0-180.0)	96.48(0.0-180)	20.84 (0.0-67.50)

2

Table 4 – Parameter estimates from GLMMs for behavioural movement changes of *T. cristatus* in a road mitigation system.

Behaviour	**Variables**	**Model parameters**					
		β	**SE**	**Chisq**	**df**	**P-value**	**AIC**
Distance travelled per night (m)							
	Intercept	3.42	0.59				
	mitigation.tunnel	3.60	0.93	17.55	2	<0.001	556.44
Straightness (0-1)							
	Intercept	0.87	0.03				
	season.spring	0.08	0.05	4.18	1	0.04	24.66
Orientation (0º-180º)							
	Intercept	88.79	7.56				
	mitigation.tunnel	-67.95	14.77	23.69	2	<0.001	1130

For each behavioural prediction, distance travelled per night (m), straightness (0-1) and orientation (0º-180º) we present: significant factors, estimate (ß), standard error (SE), Chi-square Wald test II (*W*), p-values (bold if significant) and value of Akaike information Criterion (AIC).

1 Table S1 – Counts for adults and juvenile newts per capture position, movement and final position

2

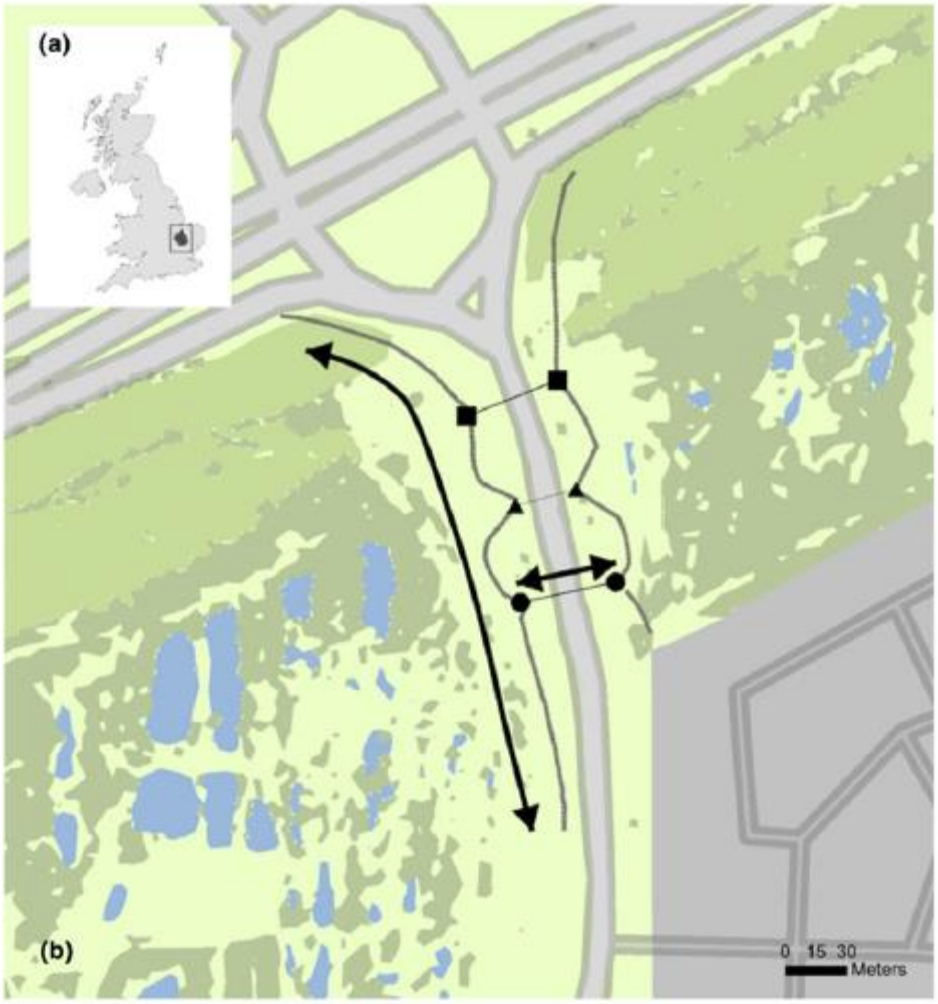
N (Total)		Capture position						Movement						Final position			
Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn			Spring		
		Fences		Tunnels		Moved		Mean distance (m)		Mean straightness (0-1)		Mean angle (o)		Mitigation	Habitat	Mitigation	Habitat
65	12	13	11	3	1	14	1	3.01	0.4	0.68	0.16	113.99	16.19	19	35	2	1
38	34	4	33	5	1	9	7	1.81	1.3	0.55	0.33	49.66	31.06	10	23	11	1
37	8	9	8	2	0	8	0	3.4	0.14	0.65	0.08	67.49	20.83	8	21	2	0
46	36	9	34	2	2	9	12	2.83	1.78	0.71	0.54	55.57	55.07	9	26	13	6
50	17	15	14	5	3	13	9	4.92	1.89	0.72	0.65	58.4	68.33	15	23	11	1
44	0	12	0	4	0	13	0	4.48	0	0.54	0	114.81	0	17	17	0	0
280	107	62	100	21	7	66	29										

N (juveniles)		Capture position						Movement						Final position			
Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn			Spring		
		Fences		Tunnels		Moved		Mean distance (m)		Mean straightness (0-1)		Mean angle (o)		Mitigation	Habitat	Mitigation	Habitat
49	7	34	6	15	1	40	2	4.04	0.79	0.63	0.27	89.16	48.57	9	31	1	1
29	17	23	16	6	1	23	5	2.86	0.57	0.68	0.29	74.18	21.18	2	22	5	0
26	8	18	8	8	0	21	2	3.6	0.43	0.69	0.25	92.86	45	3	18	2	0
35	17	23	15	12	2	26	7	3.99	1.37	0.77	0.36	81.31	39.71	5	21	6	1
30	5	16	3	14	2	25	3	5.86	0.78	0.48	0.55	72.9	90	4	21	3	0
28	0	20	0	8	0	21	0	4.06	0	0.72	0	116.03	0	6	15	0	0
197	54	134	48	63	6	156	19										

3

4

Figures


(a)
(b)
0 15 30
Meters
N
ACO underpass entrance
Southern underpass entrance
Northern underpass entrance
Fence system
Roads
Transects direction

(c)

Fig. 1 - Study area and field work transects. (a) Location of the study area in Peterborough, UK (b) transects surveyed along the mitigation system (black arrow lines with black arrows indicating direction) with location of fences (east/west sides) and tunnel entrances (symbols) (c) southern underpass entrance and fence at Hampton Nature Reserve.

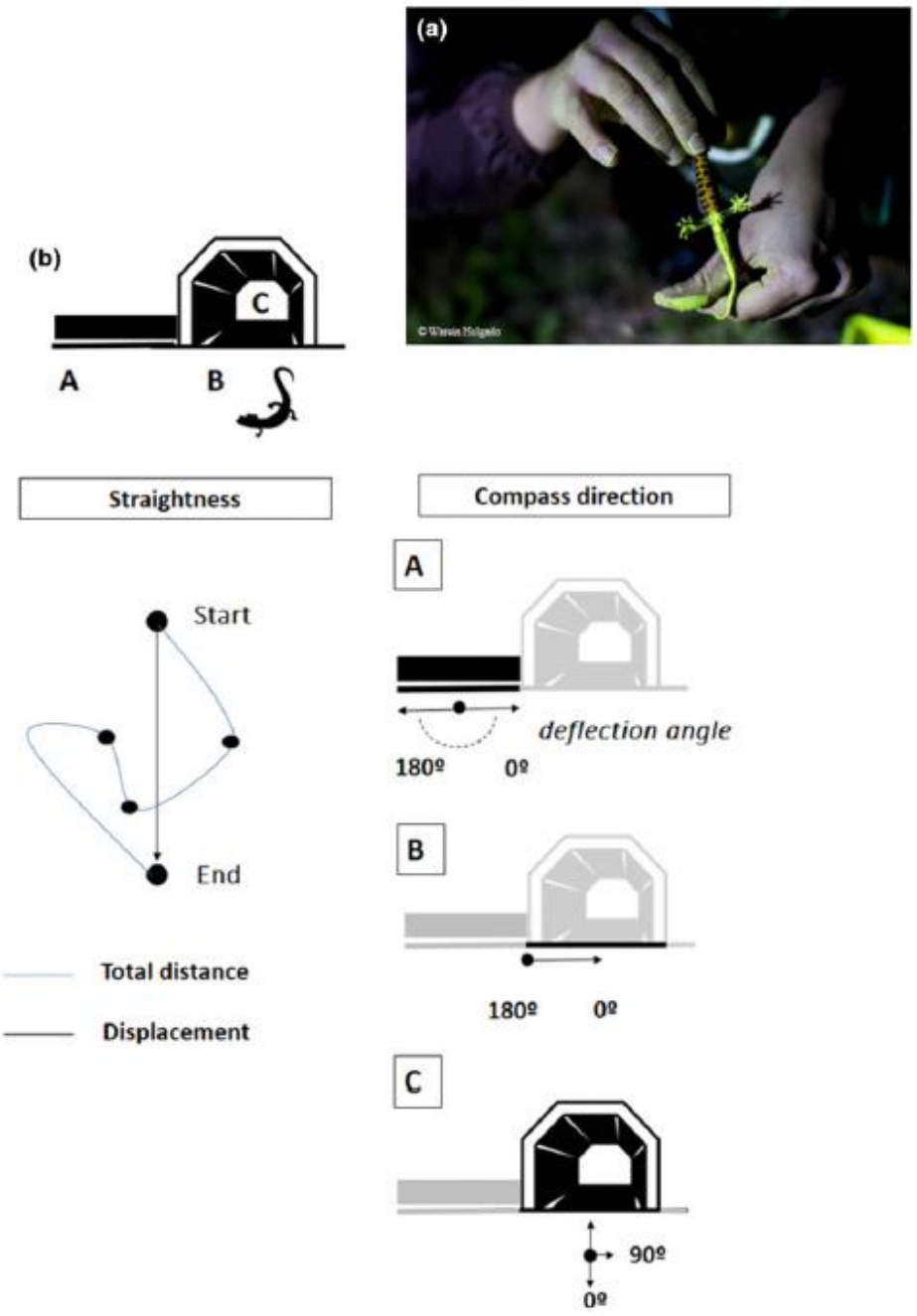


Fig. 2 – *Triturus cristatus* movement data collection. (a) Individual newt dipped in yellow fluorescent powder; (b) deflection angle ranged from 0 to 180 for three positions in the system, with 0 indicating movement directly towards the tunnel and 180 indicating movement directly away

from the tunnel. Smaller dots on the pathway diagram represent change of direction.

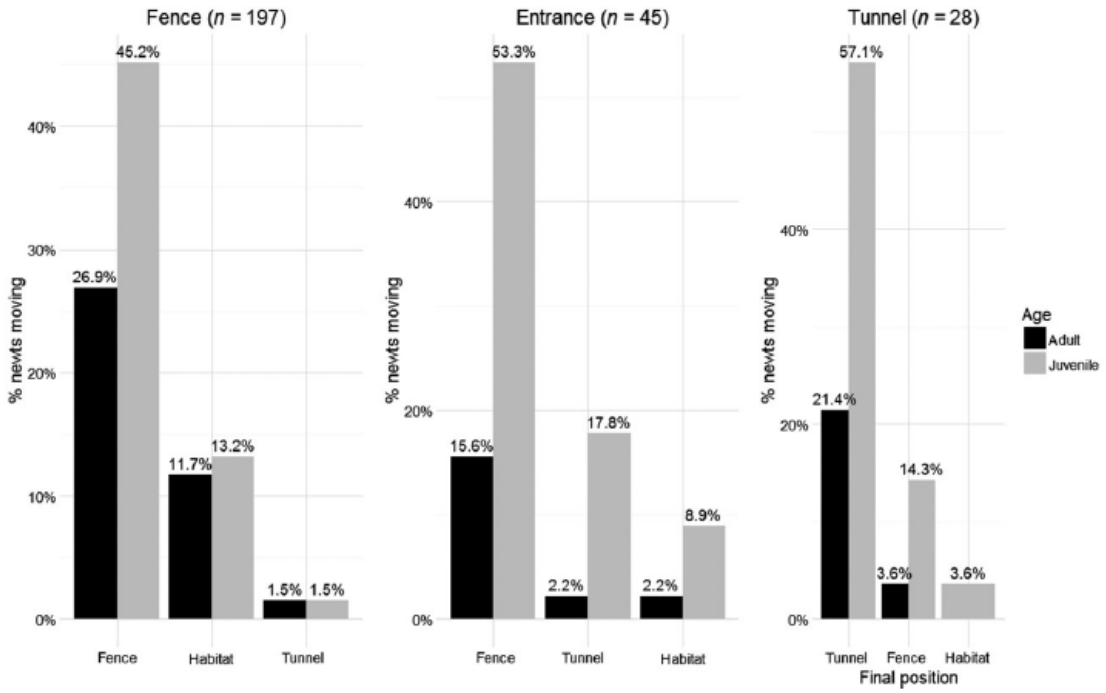


Fig. 3 – Percentages of newts (adults and juveniles) by final position (fences, tunnels or habitat) for each initial point of capture in the system

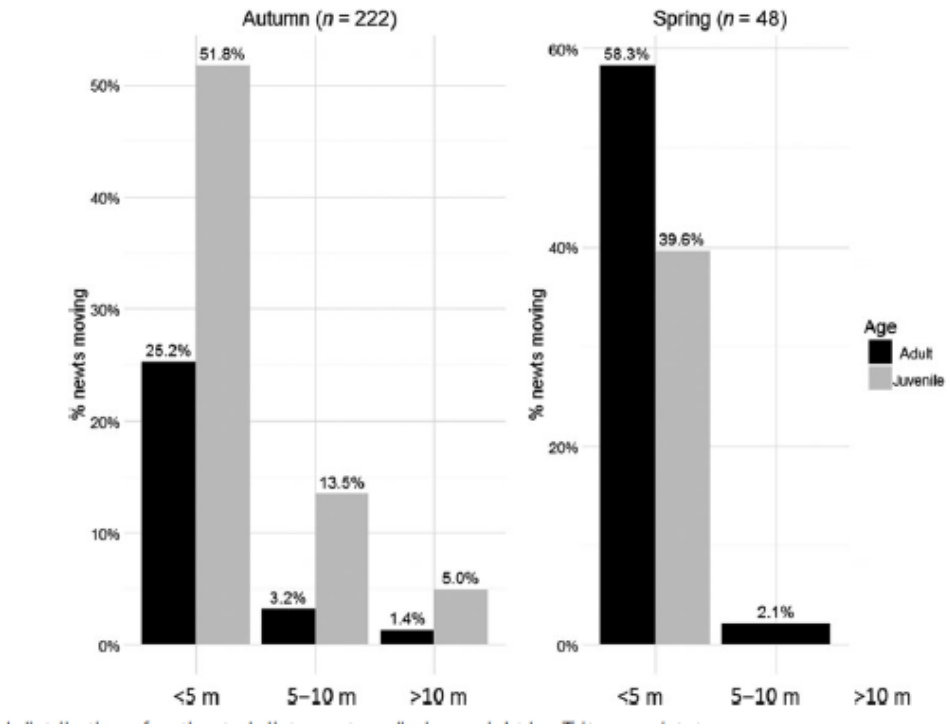


Fig. 4 – Seasonal distribution of estimated distance travelled per night by newts.

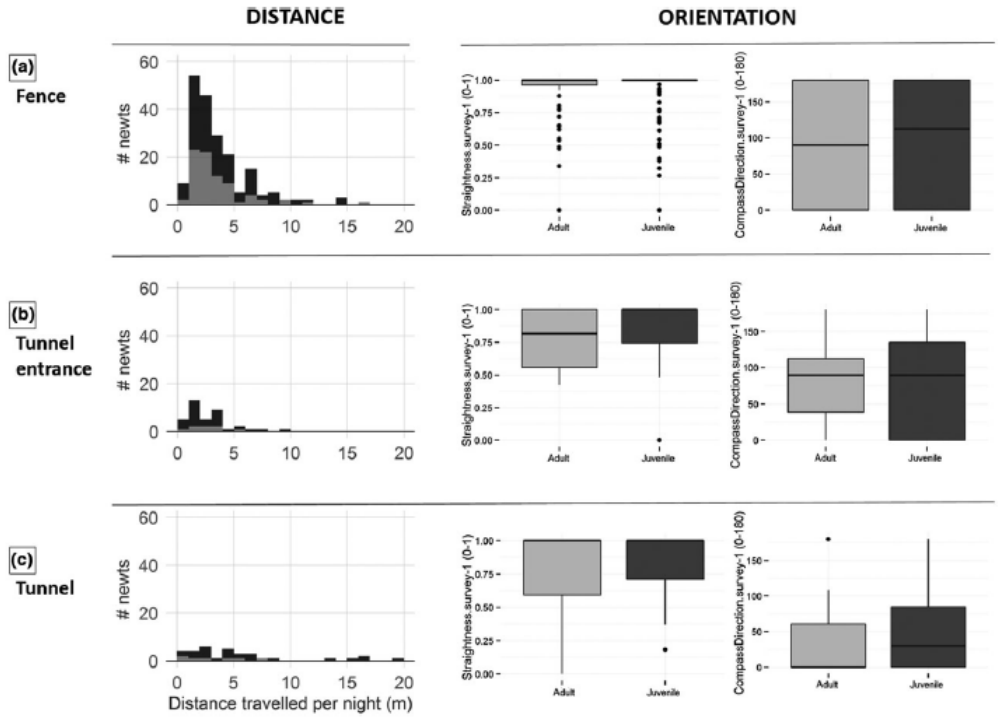


Fig. 5 – Movement behaviours of adult ($n_{females}$ = 35 and n_{males} = 44; colour: light grey) and juvenile (n = 191; colour: dark grey) *Triturus cristatus* captured at three points along the mitigation system (n_{fence} = 197; $n_{entrance}$ = 45 and n_{tunnel} = 28). Distance travelled, straightness index (0-1) and orientation relative to the tunnel were estimated per life stage class for both survey seasons.